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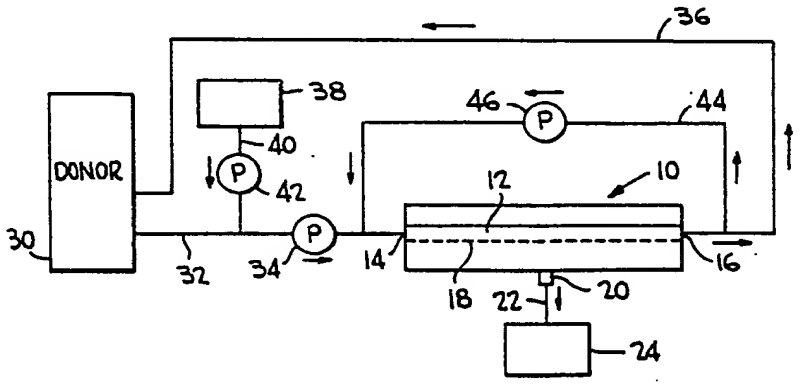

(57) Abstract

A blood cell-containing liquid suspension is separated into a cell-containing fraction and a cell-free fraction by filtration. The suspension, under pressure, is conducted in laminar flow across the surface of a microporous membrane (18, 118) along a flow path (12, 112) which is substantially parallel to the upstream side of the membrane (18, 118), the cell-containing fraction being recovered from the outlet end (16, 116) of the flow path (12, 112) and the cell-free fraction being recovered as filtrate (24, 124). The process is carried out under conditions providing a high filtration rate per area of membrane (18, 118) without causing damage to the blood cells. This is done by controlling the membrane wall shear rate of the suspension along the flow path (12, 112) so that such shear rate is sufficiently high to induce axial cell migration and inhibit interactions of the cells with the membrane surface (18, 118) at the requisite pressure conditions. Such shear rate is also maintained sufficiently low so as not to itself induce damage of the cells. Useful applications of the process include the separation of plasma from whole blood in a continuous flow plasma-pheresis procedure, and the removal of cryoprotective agents from previously frozen, thawed preparations of red blood cells, white blood cells, or platelets.

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PROCESS FOR SEPARATING BLOOD CELL-CONTAINING LIQUID SUSPENSIONS BY FILTRATION

BACKGROUND OF THE INVENTION

This invention relates to the fractionation of blood cell-containing liquid suspensions and, more particularly, to a process for effecting such fractionation by filtration through a microporous membrane.

Certain highly desirable blood processing procedures require the ability to effect an efficient separation of a liquid suspension of blood cellular components into a cellular component-containing fraction and a cellular component-free liquid fraction without causing damage to the cellular components. For example, the preservation of red blood cells, white blood cells or platelets which have been separated from whole blood for future use in transfusions, can be effectively achieved by freezing a prepared suspension of the blood cells in an electrolyte solution containing a suitable concentration of a cryoprotective agent, such as glycerol or dimethyl sulfoxide. Since the concentration of the cryoprotective agent required for the freezing procedure is well above physiologically tolerable levels, the prepared blood cell suspension must be fractionated subsequent to thawing and prior to use so as to remove the cryoprotective agent therefrom or at least to reduce its concentration in the suspension to a physiologically tolerable level. Two techniques are currently available for effecting such fractionation, one based upon the reversible agglomeration of blood cells in the presence of carbohydrates, and the other

upon various centrifugation procedures.

The problems associated with the removal of cryprotective agents has been one of the major obstacles standing in the way of more extensive clinical use of frozen cells.

In the field of red cell freezing, various advantages have been cited for promoting the use of this product. They include a possible reduction in hepatitis transmission, a reduction in transmission of undesirable antigens and antibodies, and most important, a prolonged storage period permitting accumulation of "rare red cells" blood for autologous transfusion, and stockpiling for use during shortages. Current technology can be used to achieve these goals but a more simple and efficient system is needed.

Platelets frozen storage is desirable in order to reduce outdating and allow the provision of "matched" or autologous cells. Techniques currently in use are not satisfactory and the microporous system may be suitable for such an application. Similarly, white cell storage is a problem and transfusion of unfrozen products are still basically experimental. However, it is expected that utilization will increase, and that frozen storage will be needed for their efficient management.

Another highly desirable blood processing procedure involving the separation of a liquid suspension of blood cellular components into a cellular component-containing fraction and a cellular component-free liquid fraction, is plasmapheresis. Plasmapheresis is defined as the process of removal of whole blood from the body of a blood donor by venesection, separation of its plasma portion, and reintroduction of the cellular portion into the donor's bloodstream. The cell-free plasma thus collected may either be used directly for patient care or further processed into specific plasma derivatives for clinical use. The return of the cellular components to the donor provides this plasma collection procedure with the advantage that it enables

donations by the donor at more frequent intervals. In addition to its use for plasma collection, plasmapheresis also has therapeutic implications in plasma exchange procedures for the treatment of various clinical disorders.

Currently, the most efficient and commonly employed techniques for carrying out the plasmapheresis procedure utilize "batch" centrifugation systems for effecting the separation of the cell-free plasma from the whole blood. The most serious drawback with these currently used techniques is the relatively long period of donor time which they require, typically ranging from one to one-and-a-half hours or more for collecting 500 ml of cell-free plasma. Such long period of donor time tends to have a detrimental effect upon the recruitment of volunteer donors and upon the overall cost-effectiveness of the plasmapheresis procedure.

Techniques for the separation of cell-free plasma from whole blood by filtration through a microporous membrane have previously been proposed. For example, in U. S. Patent No. 3,705,100, issued December 5, 1972, to Blatt, et al, there is disclosed a blood fractionating process and apparatus wherein whole blood is conducted in laminar flow across the surface of a microporous membrane along a flow path which is substantially parallel to the upstream side of the membrane under pressure conditions at the inlet and outlet ends of the flow path sufficient to maintain the laminar flow and to provide a filtration driving force from the upstream side to the downstream side of the membrane. Cell-free plasma is recovered as filtrate from the downstream side of the membrane, and the cellular component-containing fraction is recovered from the outlet end of the flow path. The patent teaches that one embodiment of the process and apparatus disclosed therein is capable of separating approximately 3.0 to 3.4 ml of plasma from a 10 ml sample of fresh blood of normal hematocrit in a filtering time of 15 to 20 minutes.

While such filtering capacity may be adequate for the in vitro processing of relatively small amounts of plasma for subsequent physical, chemical or clinical analyses, it obviously would not be sufficient for practical utility in plasmapheresis, where the objective is to collect 500 ml of cell-free plasma in certainly no greater and preferably substantially less than the 60 to 90 minutes required by the standard plasmapheresis techniques.

In attempting to scale up the filtration process and apparatus disclosed in the Blatt, et al patent to a filtration capacity sufficient for practical utility in carrying out the plasmapheresis procedure, a number of interrelated factors must be taken into consideration. First of all, in order to minimize the total required membrane area so that the resulting filtration module will be reasonably compact in size, and in order to minimize the required period of donor time, it is most desirable to operate under conditions which will provide optimal filtrate flux, i.e., filtration rate per area of membrane. Since, in certain cases, the filtrate flux will be governed primarily by the transmembrane pressure, i.e., the pressure differential between the upstream and downstream sides of the membrane providing the filtration driving force, the transmembrane pressure should be maintained sufficiently high so as to maximize the filtrate flux. However, too high a transmembrane pressure will cause the blood cellular components to be forced to the membrane surface and interact therewith, leading to irreversible damage or hemolysis of the cells or possibly even to plugging of the membrane pores. Proper control of the transmembrane pressure so as to provide optimal filtration rate per area of membrane without causing damage to the cellular components is further complicated by the pressure drop from the inlet end to the outlet end of the blood flow path, which causes corresponding variations in the transmembrane pressure through the system. A relatively high

pressure drop could lead to a very low transmembrane pressure in the outlet region. Thus, in order to insure that the transmembrane pressure in the outlet region will be maintained sufficiently high for efficient operation, the transmembrane pressure in the inlet region must be correspondingly higher so as to compensate for the pressure drop through the system. Moreover, if the system is to be used for carrying out a truly continuous flow plasmapheresis procedure wherein the cellular component-containing fraction exiting from the outlet end of the filtration flow path is directly reinfused into the donor's bloodstream, a further factor influencing the transmembrane pressure through the system is the requirement that the pressure at the outlet end of the filtration flow path be at least sufficient to overcome the sum of the return venous blood pressure and the pressure drop in the return needle and tubing assembly if an accessory blood pump is to be avoided.

From the foregoing considerations, it becomes readily apparent that the problems involved in scaling up the filtration process and apparatus disclosed in the Blatt, et al patent into a practical and reliably operable clinical blood fractionating technique for use in a continuous flow plasmapheresis system are of no small magnitude.

SUMMARY OF THE INVENTION

It is, accordingly, a primary object of the present invention to provide an improved process for the separation of a liquid suspension of blood cellular components into a cellular component-containing fraction and a cellular component-free liquid fraction by filtration under pressure through a microporous membrane, which enables the transmembrane pressure providing the filtration driving force to be controllably maintained at a level providing optimal filtration rate per area of membrane without causing damage to the cellular components.

Another object of the invention is to provide a filtration process in accordance with the preceding object, which is capable of effecting the separation of cell-free plasma from whole blood at a plasma flux sufficient to provide 500 ml of plasma in approximately thirty minutes.

A further object of the invention is to provide a filtration process in accordance with the preceding objects, which is capable of being utilized in a continuous flow plasmapheresis system as an improved blood separation technique requiring a substantially shorter period of donor time than that required by the standard centrifugal techniques conventionally employed in carrying out the plasmapheresis procedure.

Still another object of the invention is to provide a filtration process in accordance with the first of the foregoing objects, which is capable of being utilized as a simple, efficient and economical technique for effecting removal of cryoprotective agent from previously frozen, thawed preparations of red blood cells, white blood cells or platelets.

A still further object of the invention is to provide a relatively simple filtration process for the deglycerolization of a previously frozen, thawed glycerol-containing red blood cell suspension, which is capable of efficiently and economically reducing the glycerol concentration in the suspension from a cryoprotectively effective level to a physiologically tolerable level without causing hemolysis of the red blood cells.

The above and other objects are achieved in accordance with the present invention by means of a filtration process similar to that described in the aforementioned Blatt, et al patent, but carried out under controlled operating conditions which permit the process to be successfully scaled up to more practically useful filtration capacities. Thus, in the process of the present invention, a liquid suspension of blood cellular

components is separated into a cellular component-containing fraction and a cellular component-free liquid fraction by filtration through a microporous membrane while being conducted in laminar flow across the surface of the membrane along a flow path which is substantially parallel to the upstream side of the membrane under pressure conditions at the inlet and outlet ends of the flow path sufficient to maintain the laminar flow and to provide a filtration driving force from the upstream side to the downstream side of the membrane. The cellular component-containing fraction is recovered from the outlet end of the flow path, and the cellular component-free liquid fraction is recovered as filtrate from the downstream side of the membrane. The improvement of the present invention, which enables the transmembrane pressure providing the filtration driving force to be maintained at a level providing optimal filtration rate per area of membrane without causing lysis or damage to the cellular components, consists of controlling the membrane wall shear rate of the suspension along the flow path so that such shear rate will be sufficiently high to cause axial migration of cells and inhibit interactions of the cellular components with the membrane surface at the requisite transmembrane pressure and sufficiently low so as not to itself induce mechanical lysis or damage to the cellular components.

By properly controlling the membrane wall shear rate of the blood cell-containing liquid suspension along the flow path in accordance with the present invention, it is possible to scale up the filtration process to a filtration capacity rendering it practical for use as the blood separation technique in a continuous flow plasmapheresis system, requiring a substantially shorter period of donor time than that required by the standard centrifugal techniques conventionally employed for this purpose. For example, by operating the filtration process under the controlled conditions of the present

invention, it has been found possible to design a filtration module of reasonably compact size capable of effecting the separation of substantially hemoglobin-free and cell-free plasma from whole blood at a plasma flux sufficient to yield 500 ml of plasma in approximately thirty minutes and at pressure conditions at the outlet end of the blood flow path sufficient to enable reinfusion of the cellular component-containing fraction into the donor's bloodstream without the necessity for a specific accessory blood pump for this purpose. Furthermore, the improvement of the present invention broadens the applicability of the filtration process to also render it a relatively simple, efficient and economical technique for effecting removal of cryoprotective agent from a previously frozen, thawed preparation of blood cells. For example, when employed for the deglycerolization of a previously frozen, thawed glycerol-containing red blood cell suspension, the filtration process carried out under the controlled operating conditions of the present invention and in a continuous recirculation mode of operation, has been found to be capable of reducing the glycerol concentration in a unit of frozen red blood cells from a cryoprotectively effective level to a physiologically tolerable level in a period of approximately thirty minutes, without causing any significant hemolysis of the red blood cells.

BRIEF DESCRIPTION OF THE DRAWINGS

Other features and advantages of the present invertion will be apparent from the following detailed description of preferred embodiments accompanied by the attached drawings, in which:

Figure 1 is a schematic flow diagram of a continuous flow plasmapheresis system incorporating the improved filtration process of the present invention; and

Figure 2 is a schematic flow diagram of a system for the removal of cryoprotective agent from a previously

frozen, thawed blood cell suspension, incorporating the improved filtration process of the present invention.

DESCRIPTION OF PREFERRED EMBODIMENTS

The microporous membranes suitable for use in carrying out the filtration process of the present invention are known filter materials having holes of controlled shape and size running through their thickness and capable of effecting separation of very small particulate or molecular components from suspensions or solutions. Such microporous membranes are commercially available in various pore sizes. For example, polycarbonate microporous membranes are commercially available under the trademark NUCLEPORE from the Nuclepore Corporation, and cellulosic ester microporous membranes are commercially available from Millipore Corporation. While microporous membranes are normally supplied in thin sheet form, they can also be used in carrying out the filtration process of the present invention in other configurations, for example, hollow fibers. Suitable pore sizes found effective for filtering cell-free plasma from whole blood or cryoprotective agent from previously frozen, thawed blood cell suspensions, range broadly from about 0.2 to about 1.5 microns in diameter, and preferably from about 0.40 to about 0.60 microns in diameter.

The transmembrane pressures required for effectively carrying out the filtration process of the present invention will vary with the total effective void area of the membrane which, in turn, will be a function of both the membrane pore size and the total membrane surface area employed. Furthermore, as pointed out above, the transmembrane pressure at the inlet end of the filtration flow path will have to be sufficiently high so as to compensate for the pressure drop through the system and insure efficient operation at the outlet end of the filtration flow path. For proper control of the filtration operating conditions in accordance with the

present invention, the transmembrane pressures required for efficient operation should not be below about 50 mm Hg and should not exceed about 500 mm Hg.

For controlling the filtration operating conditions in accordance with the improvement of the present invention, the critical parameter is the wall shear rate at the membrane surface of the blood cell-containing liquid suspension along the filtration flow path. Such membrane wall shear rate is a function of both the flow rate of the liquid suspension along the filtration flow path and the geometry of the filtration flow channel, and, for rectangular flow channels, can be expressed by the relationship:

$$S=3/2 \times \frac{Q}{h^2 \times w}$$

wherein S is the membrane wall shear rate in sec^{-1}, Q is the inlet flow rate of the blood cell-containing liquid suspension in cm^3/sec, h is 1/2 of the flow channel height above the membrane surface in cm, and w is the width of the flow channel across the membrane surface in cm. Thus, the membrane wall shear rate will increase with increasing flow rates and/or decreasing flow channel dimensions.

The manner in which the membrane wall shear rate operates in accordance with the present invention as the controlling parameter for insuring optimal filtrate flux without the occurrence of lysis or damage to the cellular components, requires an understanding of the interrelationships which exist in filtration systems of this type between the membrane wall shear rate, the transmembrane pressure, the filtrate flux, and the onset of lysis of the cells. It has first of all been found that, at a constant membrane wall shear rate which is below that which would itself induce mechanical lysis of the cells, the filtrate flux will increase with increasing transmembrane pressure up to a maximum level

which will remain substantially constant with further increases in transmembrane pressure. At this maximum level of filtrate flux, there is a limited range of transmembrane pressures which will result in lysis-free operation. If the transmembrane pressure is increased beyond the upper limit of this range, it will produce lysis-causing interactions of the cells with the membrane surface. It has furthermore been found that increasing the membrane wall shear rate (while still keeping it below that which would itself induce mechanical lysis of the cells), results in an increase in such maximum level of filtrate flux obtainable, as well as an increase in both the lower and upper limits of the lysis-free range of transmembrane pressures producing such maximum level of filtrate flux. Hence, by properly correlating the membrane wall shear rate with the particular set of transmembrane pressure conditions employed, it is possible to operate at transmembrane pressures providing optimal filtrate flux while at the same time inhibiting lysis-causing interactions of the cellular components with the membrane surface which would otherwise occur at lower membrane wall shear rates.

For efficient control of the filtration operating conditions in accordance with the present invention, the membrane wall shear rate should be maintained at a minimum of about 500 sec^{-1}. It is also important to keep such shear rate sufficiently low so that it will not itself induce mechanical lysis or damage to the cellular components. The upper limit of the shear rate depends upon the particular type of cellular components in the suspension being filtered. If the cellular components consist only of red blood cells, the upper limit of the shear rate will be about 50,000 sec^{-1}. On the other hand, if the cellular components include white blood cells or platelets, the upper limit of the shear rate will be about 10,000 sec^{-1}. For most efficient operation, the membrane wall shear rate should be maintained within the range of about 500 to about 5,000 sec^{-1}.

Once the operating membrane wall shear rate has been selected so as to be properly correlated with the transmembrane pressure conditions being employed to provide optimal filtrate flux without damage to the cellular components, the selected shear rate can be achieved by proper coordination of the inlet suspension flow rate with the filtration flow channel dimensions in accordance with the relationship of these parameters defined above. If necessary, adjustment of the inlet suspension flow rate to maintain the requisite membrane wall shear rate may be provided, for example, by means of suitable pumps. For example, in carrying out a continuous flow plasmapheresis procedure wherein whole blood is transferred to the inlet end of the filtration flow path directly from a blood donor, the blood flow which can normally be obtained from an antecubital vein is about 60 ml/min. If a higher inlet blood flow rate is required for maintaining the requisite membrane wall shear rate, the blood rate from the donor may be suitably augmented by a recirculated flow system leading from the outlet end of the filtration flow path back to the inlet end thereof.

Referring now to Figure 1 of the drawings, a schematic flow diagram is provided illustrating the use of the improved filtration process of the present invention as the blood fractionating technique in a continuous flow plasmapheresis system. The system employs a filtration module 10 provided with a continuous filtration flow channel 12 extending therethrough from its inlet end 14 to its outlet end 16 across the surface of the upstream side of a microporous filtration membrane 18 disposed within the filtration module so as to form one wall of the filtration flow channel 12. The filtration module 10 is provided with a filtrate exit port 20 on the downstream side of the membrane 18. The filtrate exit port 20 is connected via conduit 22 to a filtrate collector 24.

The filtration module 10 is connected to the vein

of a blood donor 30 via a blood supply conduit 32 provided with a blood pump 34 and connected to the inlet end 14 of the filtration flow channel 12, and a blood cell return conduit 36 connected to the outlet end 16 of the filtration flow channel 12. An anti-coagulant supply container 38 is connected, via a conduit 40 provided with a pump 42, into the blood supply conduit 32 between the donor 30 and the blood pump 34. A recirculation conduit 44 provided with a pump 46 is connected at its one end into the blood cell return conduit 36 adjacent to the outlet end 16 of the filtration flow channel 12, and at its other end into the blood supply conduit 32 between the blood pump 34, and the filtration module 10 and adjacent to the inlet end 14 of the filtration flow channel 12, to thereby provide a recirculation flow loop leading from the outlet end 16 to the inlet end 14 of the filtration flow channel 12.

Before the donor is connected into the system for carrying out a continuous flow plasmapheresis procedure, the blood supply conduit 32, the filtration flow channel 12, the blood cell return conduit 36, and the recirculation conduit 34, are all first of all primed with saline solution. Thereafter, the donor is connected into the system, and whole blood is withdrawn from the donor into the blood supply conduit 32, wherein anticoagulant, pumped by pump 42 through conduit 40 from the anticoagulant supply container 38, is added thereto. The whole blood is then driven by pump 34 into the inlet end 14 of the filtration flow channel 12. As the blood flows along the filtration flow channel 12, cell-free plasma passes through the microporous membrane 18 to the downstream side thereof, while the cellular components of the blood are retained on the upstream side of the membrane. The cell-free plasma thereby separated from the blood leaves the filtration module 10 through the filtrate exit port 20 and flows through the conduit 22 into the filtrate collector 24. The cellular component-containing fraction of the blood exits from the outlet end 16 of the

filtration flow channel 12 into the blood cell return conduit 36 for reinfusion back into the donor 30. Under the action of the pump 46 in the recirculation conduit 44, a portion of the cellular component-containing fraction will be diverted from the blood cell return conduit 36 and recirculated via recirculation conduit 44 and blood supply conduit 32 back to the inlet end 14 of the filtration flow channel 12.

In carrying out the above-described plasmapheresis procedure in accordance with the present invention, the membrane wall shear rate of the blood flowing through the filtration flow channel 12 must be maintained sufficiently high to inhibit lysis-causing interactions of the blood cells with the membrane surface under the transmembrane pressure conditions existing in the filtration module under the action of the pumps 34 and 46, so as to avoid injury to the blood cells being returned to the donor and to insure that the plasma collected by the procedure is free of hemoglobin. To this end, the recirculation flow rate through the recirculation conduit 44 should be such that in combination with the flow rate of the whole blood coming from the donor, it is sufficient to maintain the requisite membrane wall shear rate. If the flow rate of the whole blood coming from the donor is alone sufficient to maintain the requisite membrane wall shear rate, then the recirculated flow may be dispensed with, in which case the entire blood cell-containing fraction exiting from the outlet end 16 of the filtration flow channel 12 would be transferred back to the donor.

Referring now to Figure 2 of the drawings, a schematic flow diagram is provided illustrating the use of the improved filtration process of the present invention in a system for the removal of cryoprotective agent from a previously frozen, thawed blood cell preparation. The system employs a filtration module 110, similar to filtration module 10 described above, provided with a continuous filtration flow channel 112

extending therethrough from its inlet end 114 to its outlet end 116 across the surface of the upstream side of a microporous filtration membrane 118 disposed within the filtration module so as to form one wall of the filtration flow channel 112. The filtration module 110 is provided with a filtrate exit port 120 on the downstream side of the membrane 118. The filtration exit port 120 is connected via conduit 122 to a filtrate collector 124.

The filtration module 110 is connected in a recirculated flow arrangement to a blood cell suspension reservoir 150 via a suspension supply conduit 152 provided with a pump 154 and connected to the inlet end 114 of the filtration flow channel 112, and a suspension return conduit 156 connected to the outlet end 116 of the filtration flow channel 112. A diluent reservoir 158 is connected into the suspension return conduit 156 via a conduit 160 provided with a pump 162.

At the start of operation, the suspension reservoir 150 contains a previously frozen, thawed suspension of blood cellular components, i.e., either red blood cells, white blood cells or platelets, in an electrolyte solution containing a cryoprotectively effective concentration of a cryoprotective agent, such as glycerol or dimethylsulfoxide. The diluent reservoir 158 contains cryoprotective agent-free electrolyte solution.

In operation, the blood cell suspension is pumped from the suspension reservoir 150 by means of pump 154 through the suspension supply conduit 152 into the inlet end 114 of the filtration flow channel 112. As the suspension flows along the filtration flow channel 112, a portion of the electrolyte solution and a portion of the cryoprotective agent pass through the microporous membrane 118 to the downstream side thereof, while the blood cells are retained on the upstream side of the membrane. The cell-free cryoprotective agent-containing filtrate leaves the filtration module 110 through the filtrate exit port 120 and passes through the conduit 122 into the filtrate collector 124. The blood

cell-containing fraction exits from the outlet end 116 of the filtration flow channel 112 into the suspension return conduit 156 through which it is returned to the suspension reservoir 150 for recirculation through the system. The recirculating fraction is diluted with additional amounts of electrolyte solution pumped by means of pump 162 from the diluent reservoir 158 through the conduit 160 and into the suspension return conduit 156, so as to at least partially compensate for the reduction in the electrolyte solution to blood cell ratio in the recirculating fraction resulting from electrolyte solution removal in the filtration module. The recirculation is carried out continuously at least until the cryoprotective agent concentration in the resulting blood cell suspension in the suspension reservoir 150 has been reduced to a physiologically tolerable level so that such suspension is ready for transfusion in humans. For example, in the case of glycerol, which is the most frequently employed cryoprotective agent, the physiologically tolerable level is about 0.1 moles per liter.

In carrying out the above-described procedure for removal of cryoprotective agent in accordance with the present invention, the membrane wall shear rate of the blood cell suspension flowing through the filtration flow channel 112 must be maintained sufficiently high to inhibit lysis-causing interactions of the blood cells with the membrane surface at the transmembrane pressure conditions existing in the filtration module under the action of the pump 154, in order to avoid injury to the blood cells. To this end, the diluent flow rate from the diluent reservoir 158 should be such that in combination with the flow rate of the recirculating fraction, it is sufficient to maintain the requisite membrane wall shear rate.

The particular design of the filtration module for use in carrying out the process of the present invention, may be varied somewhat from that indicated schematically

in the accompanying drawings, so long as the filtration flow channel dimensions are properly coordinated with the inlet suspension flow rate so as to provide the requisite membrane wall shear rate. For example, the filtration module may be designed to have a plurality of parallel filtration flow channels spaced across the width of the membrane surface, with an inlet flow distributor for dividing and directing the flow of incoming suspension to the inlet ends of the respective channels, and an outlet flow collector for reuniting the flow of outgoing suspension from the outlet ends of the respective channels.

The preferred filtration module design for use in carrying out the process of the present invention is that described and claimed in the application filed simultaneously herewith corresponding to the U. S. patent application of Barry A. Solomon and Michael J. Lysaght, Serial No. 909,459, filed May 25, 1978, entitled "FILTRATION APPARATUS FOR SEPARATING BLOOD CELL-CONTAINING LIQUID SUSPENSIONS", and incorporated herein by reference. In such filtration module, the filtration flow channels gradually and uniformly increase in width from their inlet ends to their outlet ends. Since the membrane wall shear rate of the liquid suspension flowing along the channel varies inversely with the channel width, such diverging width channel design results in such shear rate gradually and uniformly varying along the length of the flow channel, being at its highest value at the inlet end of the channel where the transmembrane pressure is also at its highest, and being at its lowest value at the outlet end of the channel where the transmembrane pressure is also at its lowest. By thus more precisely correlating the membrane wall shear rate with the transmembrane pressure conditions along the length of the filtration flow channel, such design enables better control of the filtration operating conditions. In its preferred embodiment, the filtration module is provided with a total of six parallel filtration flow channels, each of the diverging width

design, and arranged in a configuration similar to the multiple channel configuration described above, but in upper and lower sets of three channels sandwiched between two microporous membranes so that the upper membrane forms the membrane wall of the upper set of channels, and the lower membrane forms the membrane wall of the lower set of channels.

The invention is further illustrated by way of the following examples, in which the filtration module employed was designed in accordance with the above-described preferred embodiment of the filtration module of the above-identified Solomon, et al application. The filtration module had a total filtration area of 402 cm^2, divided evenly among its six filtration flow channels. Each channel had a height of 0.051 cm, an effective filtration length of 40.6 cm, a width of 1.1 cm at the inlet end of the filtration area and gradually and uniformly widening to 2.2 cm at the outlet end of the filtration area, and a filtration area of 67 cm^2. Each of the two filtration membranes employed in the filtration module was a polycarbonate microporous membrane having an average pore diameter of 0.6 microns.

EXAMPLE 1

A simulated continuous flow plasmapheresis procedure was carried out utilizing the system illustrated in Figure 1 and operated as described above, but employing a simulated donor consisting of a blood supply container connected to the donor end of the blood supply conduit 32, and a blood collection container connected to the donor end of the blood cell return conduit 36. The blood supply container was filled with freshly collected CPD anticoagulated whole human blood of normal hematocrit. The filtrate line was operated at atmospheric pressure. The inlet blood pump 34 was operated at a flow rate of 70 ml/min, and the recirculation pump 46 was operated at a flow rate of 200 ml/min, providing an inlet suspension flow rate into the filtration module of 270

ml/min, a transmembrane pressure of 180 mm Hg and a membrane wall shear rate of 2,000 sec^{-1} at the inlet end of the filtration flow channels, and a transmembrane pressure of 100 mm Hg and a membrane wall shear rate of 1,000 sec^{-1} at the outlet end of the filtration flow channels. (If the system were connected to a donor in an actual continuous flow plasmapheresis procedure, the outlet pressure of 100 mm Hg would be sufficient to enable reinfusion of the blood cell-containing fraction into the donor's bloodstream without the necessity for an accessory blood pump.)

The procedure resulted in the collection of 500 ml of plasma in the filtrate collector 24 in an operating time of approximately 30 minutes. The plasma so collected was cell-free with an acceptably low level of hemoglobin content, indicating substantially hemolysis-free operation of the system.

EXAMPLE 2

The system illustrated in Figure 2 and operated in the manner described above, was utilized for effecting the deglycerolization of a previously frozen, thawed preparation of red blood cells in a glycerol-containing electrolyte solution having a cryoprotectively effective glycerol concentration of approximately 1.4 moles per liter. The filtrate line was operated at atmospheric pressure, the diluent pump 162 was operated at a diluent flow rate of 30-40 ml/min, and the inlet suspension pump 154 was operated at an inlet suspension flow rate of 270 ml/min, providing a transmembrane pressure of 150 mm Hg and a membrane wall shear rate of 2,000 sec^{-1} at the inlet end of the filtration flow channels, and a transmembrane pressure of 70 mm Hg and a membrane wall shear rate of 1,000 sec^{-1} at the outlet end of the filtration flow channels.

The process was operated in a continuous recirculation mode until the glycerol concentration in the red blood cell suspension in the suspension reservoir 150 had been reduced to a level of about 0.1 moles per liter,

which required approximately 30 minutes. The filtrate recovered in the filtrate collector 124 contained glycerol, was cell-free, and had a free hemoglobin concentration not significantly greater than that of the original red blood cell suspension, indicating substantially hemolysis-free operation of the filtration system.

THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

Claim 1. In a process for the separation of a liquid suspension of blood cellular components into a cellular component-containing fraction and a cellular component-free liquid fraction by filtration through a microporous membrane comprising the steps of conducting said suspension in laminar flow across the surface of said membrane along a flow path which is substantially parallel to the upstream side of said membrane under pressure conditions at the inlet and outlet ends of said flow path sufficient to maintain said laminar flow and to provide a filtration driving force from said upstream side to the downstream side of said membrane, recovering said cellular component-containing fraction from said outlet end of said flow path, and recovering said cellular component-free liquid fraction as filtrate from said downstream side of said membrane, the improvement enabling said pressure conditions to be maintained at a level providing optimal filtration rate per area of membrane without causing damage to said cellular components consisting of controlling the membrane wall shear rate of said suspension along said flow path so that said shear rate will be sufficiently high to inhibit interactions of said cellular components with said membrane surface at the requisite pressure conditions and sufficiently low so as not to itself induce damage to said cellular components.

Claim 2. The process of Claim 1, wherein said shear rate is maintained at a minimum of about 500 sec^{-1}.

Claim 3. The process of Claim 2, wherein said cellular components consist of red blood cells, and said shear rate is maintained within the range of from about 500 to about 50,000 sec^{-1}.

Claim 4. The process of Claim 2, wherein said cellular components comprise white blood cells or

platelets, and said shear rate is maintained within the range of from about 500 to about 10,000 sec^{-1}.

Claim 5. The process of Claim 1, wherein said microporous membrane has a pore size from about 0.2 to about 1.5 microns in diameter, the transmembrane pressure providing said filtration driving force is maintained within the range of from about 50 to about 500 mm Hg, and said shear rate is maintained within the range of from about 500 to about 5,000 sec^{-1}.

Claim 6. The process of Claim 1, wherein said liquid suspension comprises whole blood, and said filtrate separated therefrom is plasma.

Claim 7. The process of Claim 6, wherein said whole blood is transferred to said inlet end of said flow path directly from a blood donor, and at least a portion of said cellular component-containing fraction is transferred from said outlet end of said flow path directly back to said blood donor.

Claim 8. The process of Claim 7, wherein a portion of said cellular component-containing fraction is recirculated from said outlet end of said flow path back to said inlet end of said flow path at a flow rate which in combination with that of said whole blood from the donor is sufficient to maintain the requisite membrane wall shear rate of said liquid suspension along said flow path.

Claim 9. The process of Claim 1, wherein said liquid suspension comprises a previously frozen, thawed preparation of blood cellular components in a cryoprotective agent-containing electrolyte solution, and said filtrate separated therefrom comprises a portion of said electrolyte solution and at least a portion of said cryoprotective agent sufficient to reduce the cryoprotective agent concentration in the final cellular component-containing fraction to a physiologically tolerable level.

Claim 10. The process of Claim 9, wherein said separation is effected by continuous recirculation of the cellular component-containing fraction exiting from said outlet end of said flow path, back to said inlet end of said flow path and along said flow path until the requisite amount of said cryoprotective agent has been removed therefrom, and the resulting reduction in the electrolyte solution to cellular component ratio in the recirculating fraction is at least partially compensated during the recirculation by dilution of the recirculating fraction with additional amounts of electrolyte solution at a diluent flow rate which in combination with that of said recirculating fraction is sufficient to maintain the requisite membrane wall shear rate of the suspension along said flow path.

Claim 11. The process of Claim 10, wherein said liquid suspension comprises a previously frozen, thawed preparation of red blood cells in a glycerol-containing electrolyte solution, said filtrate separated therefrom contains glycerol, and the recirculation is continued at least until the glycerol concentration in the recirculating red blood cell-containing fraction has been reduced to a level of about 0.1 moles per liter.

AMENDED CLAIMS
(received by the International Bureau on 22 October 1979 (22.10.79))

Claim 1. In a process for the separation of a liquid suspension of blood cellular components into a cellular component-containing fraction and a cellular component-free liquid fraction by filtration through a microporous membrane which is permeable to blood proteins and impermeable to blood cellular components comprising the steps of conducting said suspension in laminar flow across the surface of said membrane along a flow path which is substantially parallel to the upstream side of said membrane under pressure conditions at the inlet and outlet ends of said flow path sufficient to maintain said laminar flow and to provide a filtration driving force from said upstream side to the downstream side of said membrane, recovering said cellular component-containing fraction from said outlet end of said flow path, and recovering said cellular component-free liquid fraction as filtrate from said downstream side of said membrane, the improvement enabling said pressure conditio to be maintained at a level providing optimal filtration rate per area of membrane without causing damage to said cellular components consisting of controlling the membrane wall shear rate of said suspension along said flow path so that said shear rate will be sufficiently high to inhibit interactions of said cellular components with said membrane surface at the requisite pressure conditions and sufficiently low so as not to itself induce damage to said cellular components.

Claim 2. The process of Claim 1, wherein said shear rate is maintained at a minimum of about 500 sec^{-1}.

Claim 3. The process of Claim 2, wherein said cellular components consist of red blood cells, and said shear rate is maintained within the range of from about 500 to about 50,000 sec^{-1}.

Claim 4. The process of Claim 2, wherein said cellular components comprise white blood cells or

platelets, and said shear rate is maintained within the range of from about 500 to about 10,000 sec^{-1}.

Claim 5. The process of Claim 1, wherein said microporous membrane has a pore size from about 0.2 to about 1.5 microns in diameter, the transmembrane pressure providing said filtration driving force is maintained within the range of from about 50 to about 500 mm Hg, and said shear rate is maintained within the range of from about 500 to about 5,000 sec^{-1}.

Claim 6. The process of Claim 1, wherein said liquid suspension comprises whole blood, and said filtrate separated therefrom is plasma.

Claim 7. The process of Claim 6, wherein said whole blood is transferred to said inlet end of said flow path directly from a blood donor, and at least a portion of said cellular component-containing fraction is transferred from said outlet end of said flow path directly back to said blood donor.

Claim 8. The process of Claim 7, wherein a portion of said cellular component-containing fraction is recirculated from said outlet end of said flow path back to said inlet end of said flow path at a flow rate which in combination with that of said whole blood from the donor is sufficient to maintain the requisite membrane wall shear rate of said liquid suspension along said flow path.

Claim 9. The process of Claim 1, wherein said liquid suspension comprises a previously frozen, thawed preparation of blood cellular components in a cryoprotective agent-containing electrolyte solution, and said filtrate separated therefrom comprises a portion of said electrolyte solution and at least a portion of said cryoprotective agent sufficient to reduce the cryoprotective agent concentration in the final cellular component-containing fraction to a physiologically tolerable level.

Claim 10. The process of Claim 9, wherein said separation is effected by continuous recirculation of the cellular component-containing fraction exiting from said outlet end of said flow path, back to said inlet end of said flow path and along said flow path until the requisite amount of said cryoprotective agent has been removed therefrom, and the resulting reduction in the electrolyte solution to cellular component ratio in the recirculating fraction is at least partially compensated during the recirculation by dilution of the recirculating fraction with additional amounts of electrolyte solution at a diluent flow rate which in combination with that of said recirculating fraction is sufficient to maintain the requisite membrane wall shear rate of the suspension along said flow path.

Claim 11. The process of Claim 10, wherein said liquid suspension comprises a previously frozen, thawed preparation of red blood cells in a glycerol-containing electrolyte solution, said filtrate separated therefrom contains glycerol, and the recirculation is continued at least until the glycerol concentration in the recirculating red blood cell-containing fraction has been reduced to a level of about 0.1 moles per liter.

STATEMENT UNDER ARTICLE 19

Claim 1 in the replacement sheet differs from originally filed claim 1 in the following respect:

The modified claim specifies that filtration occurs through a semipermeable membrane which is permeable to blood proteins and impermeable to blood cellular components.

FIG. 1

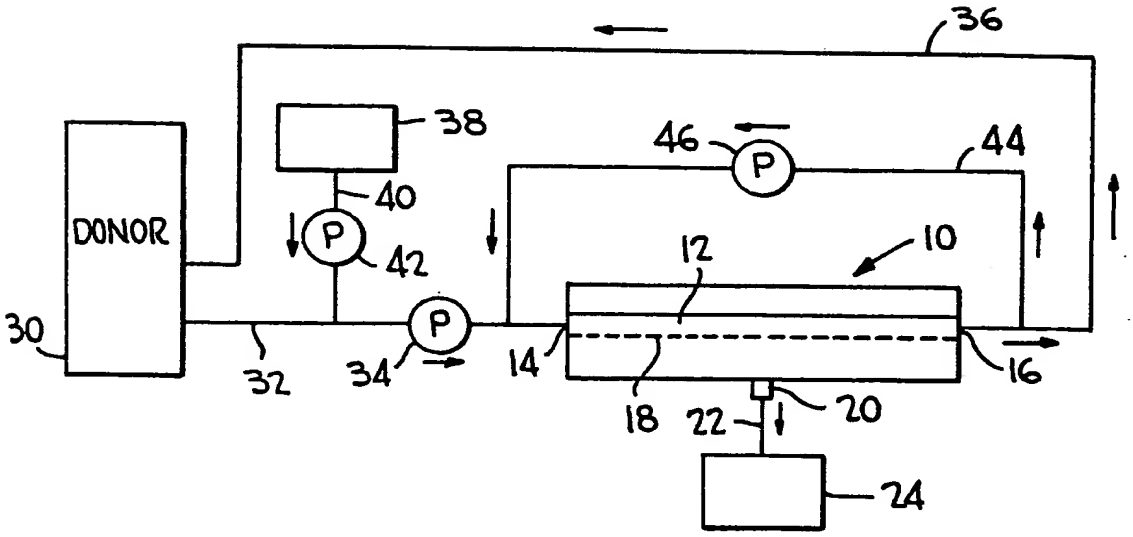
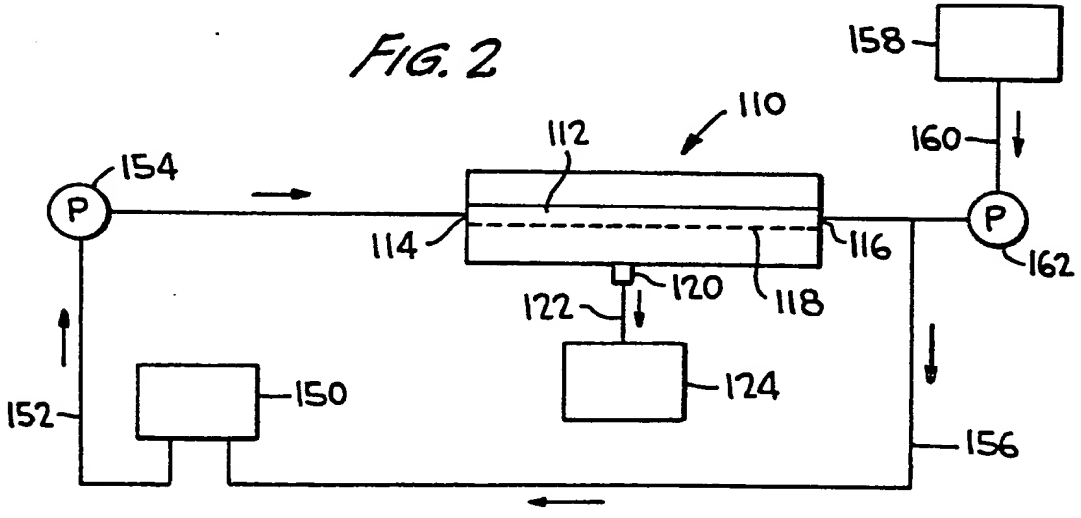


FIG. 2



INTERNATIONAL SEARCH REPORT

International Application No PCT/US79/00358

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

INT. CL. B01D 13/00; A61M 5/00

U.S. CL. 210/23F; 210/195.2; 128/214E; 260/112R

WO 80/01121

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
U.S.	210/23F,195.1,321A,321B,321R,433M 260/112R,112B; 424/101 128/214R,214E

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [5]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
A,P	US,A, 3,483,867, PUBLISHED 16 DECEMBER 1978, MARKOVITZ.	1
A	US,A, 3,705,100, PUBLISHED 05 DECEMBER 1972, BLATT, ET AL.	1,6-9
A	US,A, 3,567,031, PUBLISHED 02 MARCH 1971, LOEFFLER.	1,6-9
X	N, Transactions American Society Artificial Organs, 1968, Bixler et al, The Development of a Diafiltration System For Blood Purification, pages 99-108.	1-11
X	N, The American Society for Artificial Internal Organs, 23rd Annual Meeting, Abstract published 17 March 1977, Lysaght et al, Development of a Microporous Membrane System for Continuous Flow Plasmapheresis.	1-11

* Special categories of cited documents: [15]

"A" document defining the general state of the art

"E" earlier document but published on or after the international filing date

"L" document cited for special reason other than those referred to in the other categories

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but on or after the priority date claimed

"T" later document published on or after the international filing date or priority date and not in conflict with the application, but cited to understand the principle or theory underlying the invention

"X" document of particular relevance

IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [2]
08 AUGUST 1979	20 AUG 1979
International Searching Authority [1]	**Signature of Authorized Officer** [20]
ISA/US	FRANK A. SPEAR, JR.

Form PCT/ISA/210 (second sheet) (October 1977)